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ORIGINAL ARTICLE

Antinuclear antibody titer and treatment response to peginterferon plus ribavirin for chronic hepatitis C patients

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Abstract Positive serum antinuclear antibody (ANA) is not infrequent in chronic hepatitis C virus (HCV)-infected patients. This prospective study evaluated the impact of ANA on the response to and safety of peginterferon/ribavirin combination therapy for chronic hepatitis C patients in clinical practice. We enrolled 243 consecutive patients who were treated with a 24-week regimen of peginterferon-α plus ribavirin, with a 24-week follow-up period. ANA titer was determined before antiviral treatment. The primary end-point was sustained virological response (SVR), defined as HCV RNA $<$50 IU/mL throughout the follow-up period. Overall, 187 (77.0%) patients experienced a SVR. In the 105-patient HCV genotype non-1 group, patients with ANA titer $\geq$1:80 had a significantly lower SVR rate than those with ANA titer $<$1:80 (67.7% vs. 95.8%, respectively, $p = 0.013$). In contrast, in the 138-patient HCV genotype 1 group, the SVR rate did not differ between patients with and without ANA titer $\geq$1:80. Multivariate regressive analyses showed that ANA $\geq$1:80, age and HCV RNA levels were independent factors associated with SVR in HCV genotype non-1 patients; whereas HCV RNA levels and hepatic fibrosis were prognostic predictors of SVR in HCV genotype 1 patients. The frequencies of adverse events were similar between patients with and without ANA seropositivity. Peginterferon/ribavirin combination therapy is effective and safe in ANA-positive chronic hepatitis C patients.

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A high ANA titer was a negative prognostic factor for treatment response in HCV genotype non-1 patients.

Introduction

The prevalence of various immunological phenomena, including autoimmunity and autoimmune disease, in patients exposed to the hepatitis C virus (HCV) are reportedly high [1,2]. Autoantibodies are commonly found in patients with chronic hepatitis C (CHC) [3]. The prevalence of different non-organ-specific autoantibodies (NOSA), including antinuclear antibodies (ANA), anti-smooth-muscle antibodies, antimitochondrial antibodies, anti-neutrophil-cytoplasmic antibodies, and anti-liver/kidney microsomal antibodies, were investigated before, during and after monotherapy with interferon-α (IFN) for CHC [4,5]. The former analyses of the influence of NOSA on the clearance of the HCV are controversial. Some studies have suggested that there is no difference between autoantibody-positive and autoantibody-negative patients on IFN [6,7] or IFN/ribavirin combination therapy [8]. In contrast, others have implied that there is a tendency towards worse long-term biochemical and virological responses in autoantibody-positive patients [9,10]. Recently, combination therapy with poly-ethylene-glycol-IFN (PEG-IFN) and ribavirin has produced higher sustained virological response (SVR) rates than conventional treatment with IFN and ribavirin [1,11]. Combination therapy with PEG-IFN and ribavirin has therefore become a standard therapy for CHC but, so far, no study has investigated the relevance of autoantibodies with regard to treatment response in CHC patients taking PEG-IFN and ribavirin in combination.

ANA, a marker for autoimmune liver disease and other inflammatory conditions, has been detected in 4–41% of patients with CHC infection in several studies [5,12,13]. We found that ANA seropositivity is associated with lower HCV RNA levels and advanced fibrosis in Taiwanese CHC patients [14]. Our recent studies among such patients after combination therapy with IFN and ribavirin have shown a favorable response, suggesting that viral and host immunological factors might predict therapeutic responses [15–19]. Patients who had ANA titer of $\geq$1:80 were frequently excluded from standard clinical trials; thus the impact of ANA seropositivity $\geq$1:80 on the safety and efficacy of the treatment of CHC patients is unclear. We therefore conducted a prospective study to evaluate the impact of ANA on the response and safety to PEG-IFN/ribavirin in CHC patients and to identify clinical, biochemical or immunological features that can predict the responses to antiviral treatment in ANA-positive patients in clinical practice.

Materials and methods

Patients

Individuals who were eligible for the study were previously untreated Taiwanese CHC patients, aged 20–70 years. All patients: (1) were seropositive for HCV antibodies (anti-HCV) with serum HCV RNA detected by polymerase chain reaction for at least 6 months; (2) underwent a liver biopsy consistent with CHC within 6 months of entering the study; and (3) displayed elevated serum alanine aminotransferase (ALT) levels, defined as $\geq$1.5 times the upper limit of the normal range for at least two measurements within the 6 months preceding the trial entry. Patients with hepatitis B surface antigen (HBsAg), HIV infection, decompensated cirrhosis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, α1-antitrypsin deficiency, a current or past history of alcohol abuse ($\geq$20 g daily), previous liver transplantation or evidence of hepatocellular carcinoma were excluded from the study. All subjects were thoroughly screened for autoimmune diseases during detailed physical examination and history taking; none of our patients had received treatment for previous or present systemic autoimmune diseases involving multiple diverse organs and tissues (systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndromes, etc.). The liver histology was assessed by one pathologist who was unaware of the ANA status of the individual biopsies and all other clinical information.

Study design

The Bureau of National Health Insurance in Taiwan is now reimbursing HCV treatment using 24-week combination therapy for all genotypes. Patients who enrolled were prescribed peginterferon (PEG) α-2b (PEG-Intron, Schering-Plough Inc., Kenilworth, NJ) at a dose of 80–100 μg/week (1.5 μg/kg/week) subcutaneously, or PEG α-2a (PEGASYS, Hoffmann-La Roche, Basel, Switzerland) at a dose of 180 μg/week subcutaneously, plus oral ribavirin 1000–1200 mg daily in two divided doses for 24 weeks. The doses of ribavirin were based on body weight, with 1000 mg for patients $\leq$75 kg and 1200 mg for patients $>$75 kg. All patients were monitored for a further 24 weeks after the treatment ended. They had biweekly outpatient visits during the first month and monthly visits throughout the remaining treatment and 24-week follow-up periods. At each visit, there was a physical examination, and adverse events were recorded. Liver function, complete blood count and serum creatinine level were evaluated at each visit. HCV RNA was tested every 12 weeks for 48 weeks. The disease activity grade and stage of fibrosis were quantitatively scored according to the histological activity index [20]. This study was approved by the Kaohsiung Medical University Hospital Ethics Committee in accordance with the Declaration of Helsinki, and informed consent was obtained from all patients who enrolled.

Dose modifications

The adverse event profiles of PEG-IFN/ribavirin were graded as mild, moderate, severe or potentially life-threatening.

The dose of PEG-IFN was decreased by 50% and the dose of ribavirin was lowered to 600 mg/day when severe adverse events occurred or when laboratory results showed:

- hemoglobin <9 g/dL in patients with no cardiac disease;
- hemoglobin decrease >2 g/dL in subjects with cardiac disease;
- neutrophils <750/mm^3; or
- a platelet count <50,000/mm^3.

Full doses could be resumed when the event was resolved. If the event persisted, both drugs were discontinued. Therapy was permanently discontinued if life-threatening events occurred or when laboratory results showed hemoglobin <7.5 g/dL in subjects with no cardiac disease, hemoglobin <12 g/dL in subjects with cardiac disease after 4 weeks of dose reduction, neutrophils <500/mm^3, a platelet count <30,000/mm^3 or serum creatinine >2.0 mg/dL.

Laboratory tests

Anti-HCV was detected with commercially-available third-generation enzyme-linked immunosorbent assay kits (enzyme-immunoassay, Abbott Laboratories, North Chicago, IL). Serum HBsAg was assayed using commercially-available kits (General Biological HBsAg radioimmunoassay, General Biological Cooperation, Hsinchu, Taiwan). Aspartate aminotransferase and ALT were measured on a multichannel autoanalyzer. ANA and anti-smooth muscle antibody (SMA) were searched for using the indirect immunofluorescence on Hep-2 cells method (Medical Biological Lab, Tokyo, Japan) with an initial dilution of 1:20, following standard protocols [21,22]. Positive reactions were titrated by double dilution to the end-point. Serum reactivity at a dilution of at least 1:80 was considered a positive result for ANA in the present study.

Serum HCV RNA before treatment, at the end of treatment, and 12 and 24 weeks after therapy was determined by standardized automated qualitative polymerase chain reaction (Cobas Amplicor Hepatitis C Virus Test, version 2.0, Roche Diagnostics, Branchburg, NJ; detection limit, 50 IU/mL). HCV genotypes 1a, 1b, 2a, 2b and 3a were determined by amplification of the core region using genotype-specific primers [23]. Serum HCV RNA levels at baseline and during treatment week 12 were measured using the branched DNA assay (Versant HCV RNA 3.0, Bayer, Tarrytown, NJ; quantification limit, 615 IU/mL).

Assessment of efficacy

SVR was defined as clearance of serum HCV RNA by the end-of-treatment and throughout the follow-up period. Those who had detectable HCV RNA either at end-of-treatment or throughout the follow-up period were defined as non-SVR.

Statistical analyses

Frequency was compared between groups using the chi-square test, with Yates's correction, or with Fisher's exact test. Group means, presented as mean values ± standard deviation (SD), were compared using the student t test, or the nonparametric Mann-Whitney test, when appropriate. Serum HCV RNA levels were expressed as mean ± SD after logarithmic transformation of the original values. Stepwise logistical regression was used to analyze which variables were independent factors related to positive ANA. The procedures were performed using the SPSS 12.0 statistical package (SPSS, Chicago, IL). All statistical analyses were based on two-sided hypothesis tests with a significance level of $p < 0.05$.

Results

From December 2002 to December 2005, 243 consecutive patients (153 men and 90 women, aged 20—70 years; mean age, 51.0 ± 10.9) with a history of antiviral treatment of >20 weeks were included in the final analysis. The baseline characteristics of all 243 patients with CHC are shown in Table 1. Of these, 138 patients had HCV genotype 1 (HCV-1)

Table 1 Basic demographic and virological features of 243 chronic hepatitis C patients.

Feature	Value
Age[a] (years)	51.0 ± 10.9
Sex	
Male	153
Female	90
AST[a] (IU/L)	113.4 ± 68.7
ALT[a] (IU/L)	181.1 ± 125.9
Total serum globulin (N = 138) (mg/dl)	3.62 ± 0.58
HCV RNA level[a] (log IU/mL)	5.33 ± 0.95
HCV genotypes	
1b (*n*, %)	107 (44.0)
2a (*n*, %)	85 (35.0)
2b (*n*, %)	13 (5.3)
Mixed (*n*, %)	33 (12.6)
Unclassified (*n*, %)	5 (2.1)
HAI (N = 243)	
Necroinflammatory score[a]	5.0 ± 2.4
Fibrosis score[a]	1.6 ± 1.1
F0—2 (*n*, %)	191 (75.2)
F3—4 (*n*, %)	52 (24.8)
SVR (*n*, %)	187 (77.0)
ANA$^+$ (*n*, %)	100 (41.2)
Titer (*n* = 100)	
1:40 (*n*, %)	86 (86.0)
1:80 (*n*, %)	7 (7.0)
1:160 (*n*, %)	6 (6.0)
1:320 (*n*, %)	1 (1.0)
ASMA$^+$ (*n* = 127) (*n*, %)	23 (18.1)

Key: ALT = alanine aminotransferase, ANA = antinuclear antibody, ASMA = anti-smooth muscle antibody, AST = aspartate aminotransferase, HAI = histological activity index, HCV = hepatitis C virus, SD = standard deviation, SVR = sustained virological response.

[a] Presented as mean ± standard deviation.

infection: 107 (44.0%) were determined to have HCV-1b infection and 31 were determined to have HCV-1b combined with infection with other HCV genotypes; 105 patients had no HCV genotype-1 (HCV-non 1) infection.

One hundred (41.2%) patients had serum ANA titer >1:40, including 14 patients with serum ANA titer >1:80, seven with ANA titer >1:160 and one with ANA titer >1:320. The immunofluorescence patterns of the ANA showed 90% (90/100) speckled pattern; 7% (7/100) homogeneous pattern; and 3% (3/100) mixed pattern in the 100 patients with ANA titer >1:40. In the present study, serum ANA and SMA were both available in 127 patients; 23 (18.1%, 23/127) had serum SMA titer >1:40. Among these 127 patients, 13 patients had both serum SMA titer >1:40 and serum ANA titer >1:40; 52 patients had only serum ANA titer >1:40; and 10 patients had only serum SMA titer >1:40.

Overall, 187 (77.0%) of the 243 treated patients showed SVR to treatment. The rates of SVR were comparable between patients treated with PEG-IFN α-2a and α-2b (81.1% vs. 74.3%, $p = 0.275$); hence all of the patients were considered to be a homogeneous group, even if they received different PEG regimens. In a univariate analysis, patients who failed to achieve an SVR were significantly older (mean age, 54.0 $\pm$ 9.9 vs. 50.1 $\pm$ 11.1 years, $p = 0.017$) and had significantly higher mean HCV RNA levels (5.7 $\pm$ 0.6 log IU/mL vs. 5.2 $\pm$ 0.9 log IU/mL, $p < 0.001$), higher mean necroinflammatory scores (5.5 $\pm$ 2.2 vs. 4.8 $\pm$ 2.4, $p = 0.035$), and a higher frequency of F3–4 (33.9% vs. 17.6%, $p < 0.015$), when compared to patients with SVR (Table 2). Those with HCV-1 infection had significantly lower SVR rates (64.5% vs. 93.3%, $p < 0.001$) compared to patients with HCV-non 1 infection. There was no difference in the frequencies of different titers of ANA between non-SVR and SVR patients. In multivariate analyses, low-grade fibrosis, lower HCV RNA levels, HCV-non 1 infection and younger age were independent factors related to SVR (Table 3).

Since HCV genotype is the most important factor associated with SVR and patients with ANA titer were universally excluded from the clinical trial, we further stratified patients by genotype to evaluate the impact of ANA titers $\geq$1:80 on the response to PEG-IFN and ribavirin. In the HCV-non 1 group, patients with ANA <1:80 had a significantly higher SVR rate than those with ANA $\geq$1:80 (95.8% vs. 67.7%, respectively, $p = 0.013$). In contrast, the SVR rate did not differ between patients with and without ANA $\geq$1:80 in the HCV-1 group (Table 4). Using multivariate analyses, ANA titer $\geq$1:80, age and HCV RNA levels were independent factors related to SVR in HCV-non 1; HCV RNA levels and severity of fibrosis were independent factors related to SVR in HCV-1 patients (Table 5).

Safety profile

The rate of dose reduction of PEG-IFN or ribavirin and the frequencies of adverse events are shown in Table 6; there were no significant differences in the frequency of adverse events between ANA-positive and -negative patients. None of our patients experienced a flare-up of serum ALT levels up to five times the upper limit of normal and/or five times during treatment that required the withdrawal of

Table 2 Factors associated with sustained virological response.

Factor	Total	SVR ($n = 187$)	Non-SVR ($n = 56$)	p
Sex				
Female (n, %)	90	67 (35.8)	23 (41.1)	0.529
Male (n, %)	153	120 (64.2)	33 (58.9)	
Age[a] (y)	243	50.1 $\pm$ 11.1	54.0 $\pm$ 9.9	0.017
AST[a] (IU/L)	243	114.9 $\pm$ 73.1	108.1 $\pm$ 51.2	0.67[b]
ALT[a] (IU/L)	243	189.5 $\pm$ 134.1	153.3 $\pm$ 88.8	0.06[b]
HCV genotype				
HCV-1 (n, %)	138	89 (47.6)	49 (87.5)	<0.0001
HCV-non 1 (n, %)	105	98 (52.4)	7 (12.5)	
HCV RNA levels[a] (log IU/mL)	243	5.2 $\pm$ 0.9	5.7 $\pm$ 0.6	<0.0001
Necroinflammatory score[a]	243	4.8 $\pm$ 2.4	5.5 $\pm$ 2.2	0.035[b]
Fibrosis score[a]	243	1.5 $\pm$ 1.1	2.0 $\pm$ 1.2	0.004[b]
F0–2 (n, %)	191	154 (82.4)	37 (66.1)	0.015
F3–4 (n, %)	52	33 (17.6)	19 (33.9)	
ANA titer				
$\geq$1:40 (n, %)	100	75 (40.1)	25 (44.6)	0.643
<1:40 (n, %)	143	112 (59.9)	31 (55.4)	
$\geq$1:80 (n, %)	14	10 (5.3)	4 (7.1)	0.743
<1:80 (n, %)	229	177 (94.7)	52 (92.9)	

Key: ALT = alanine aminotransferase, AST = aspartate aminotransferase, HAI = histological activity index, HCV = hepatitis C virus; HCV-1 = with HCV genotype 1 infection; HCV-non 1 = without HCV genotype 1 infection, SD = standard deviation, SVR = sustained virological response.

[a] Presented as mean $\pm$ SD.

[b] Using nonparametric Mann-Whitney test.

Table 3 Stepwise logistic regression analysis of factors significantly associated with sustained virological response in 243 patients with chronic hepatitis C infection.

	Comparison	Odds ratio (95% CI)	*p*
Dependent variable: SVR			
Independent variable			
HCV genotype	HCV-1 = 0, HCV-non 1 = 1	9.469 (3.631–24.698)	<0.001
Age	per 1-yr increase	0.961 (0.926–0.997)	0.034
Fibrosis	F3–4 = 1, F0–2 = 0	0.298 (0.118–0.755)	0.011
HCV RNA level	per 1-log increase	0.427 (0.262–0.696)	0.001

Key: ANA = antinuclear antibody, CI = confidence interval, HCV = hepatitis C virus, HCV-1 = with HCV genotype 1 infection, HCV-non 1 = without HCV genotype 1 infection, SVR = sustained virological response.

combination therapy. Of the seven patients with high ANA titers (six patients with ANA titer ≥1:160 and one with ≥1:320) none suffered from a flare-up of serum ALT levels.

Discussion

We herein present the first large-scale study undertaken to prospectively evaluate the impact of ANA on the efficacy of 24-weeks of PEG-IFN/ribavirin combination therapy in a group of Taiwanese CHC patients in clinical practice. We have demonstrated that the presence of ANA seropositivity did not influence the efficacy of treatment in patients with HCV-1 infection. An ANA titer ≥1:80, however, adversely affected the SVR in patients with HCV-non 1 infection. We have shown that CHC patients with an ANA titer of up to 1:320 might be treated safely.

Although the 2002 National Institutes of Health consensus statement concludes that HCV-1 infections require PEG-IFN/ribavirin therapy for 48 weeks and HCV-2 or -3 for 24 weeks [24], the Bureau of National Health Insurance in Taiwan is now reimbursing HCV treatment using 24-week combination therapy for all genotypes. A 24-week regimen of PEG-IFN/ribavirin can achieve an SVR rate of 50–65% for HCV-1 patients in Taiwan [25–27]. A number of factors have been considered in terms of their potential to predict the response to combination therapy, including infection with HCV-1, high levels of viremia and the presence of advanced fibrosis, which have been reported to be associated with a relatively poor response [28–30]. These results are compatible with our present study, showing that advanced fibrosis and higher HCV RNA level suggest poor treatment efficacy.

Although treatment with PEG-IFN and ribavirin for 24 weeks could achieve a SVR rate of 80–93% for HCV-non 1 patients, a number of the "easy-to-treat" patients remained refractory to the currently recommended regimen [1,11,17,24,31–33]. It is, thus, important to find the potential prognostic factors associated with non-SVR in the refractory subgroup. The current study demonstrates that HCV-non 1 patients with ANA titer <1:80 had a significantly higher SVR rate than those with ANA titer ≥1:80. These results suggest that evaluation of ANA might be helpful in predicting treatment response for HCV-non 1 patients. Nevertheless, because of the limited number of cases in the subgroup of the current study, whether a relatively difficult-to-treat HCV-non 1 patient subgroup can benefit from a longer duration of treatment according to the ANA titer needs further large-scale study.

Various factors including advancing age, genetic predisposition, environmental agents, estrogen-androgen balance, chronic infections and neoplasm are associated with the presence of serum ANA [34]. The immune mechanism of liver damage is still unclear. Czaja and Carpenter [35], however, identified a subset of CHC patients with an autoimmune pattern of liver histology characterized by plasma cell infiltrates. Yee and coworkers also observed increased plasma cells in the liver biopsies of ANA-positive individuals compared with ANA-negative individuals [6]. The increased plasma cells might be a marker for B-cell polyclonal activity with a secondary clinical manifestation of increased serum immunoglobulin with or without autoantibody production [6,35]. These observations indicate an association between ANA and host immune status in the liver. Williams and coworkers observed that the presence of autoantibodies in HCV is associated with increased age and

Table 4 Comparisons of sustained virological response associated with antinuclear antibody in chronic hepatitis C patients with and without genotype 1 hepatitis C virus infection.

	HCV-1 (*n* = 138)			HCV-non 1 (*n* = 105)		
	ANA-positive (*n* = 5)	ANA-negative (*n* = 133)	*p*	ANA-positive (*n* = 9)	ANA-negative (*n* = 96)	*p*
SVR (*n*, %)	4 (80.0)	85 (63.9)	0.416	6 (67.7)	92 (95.8)	0.013
Non-SVR (*n*, %)	1 (20.0)	48 (36.1)		3 (33.3)	4 (4.2)	

Key: ANA = antinuclear antibody, ANA-positive = titers ≥1:80, ANA-negative = titers <1:80, HCV = hepatitis C virus, HCV-1 = with HCV genotype 1 infection, HCV-non 1 = without HCV genotype 1 infection, SVR = sustained virological response.

Table 5 Stepwise logistic regression analysis of factors significantly associated with sustained virological response in patients with and without genotype 1 hepatitis C virus infection.

Dependent variable	Independent variable	Comparison	Odds ratio (95% CI)	*p*
SVR for patients with HCV-1 infection				
	HCV RNA level	per 1-log increase	0.487 (0.294—0.806)	0.005
	fibrosis	F3—4 = 1, F0—2 = 0	0.268 (0.092—0.781)	0.016
SVR for patients with HCV-non 1 infection				
	age	per 1-yr increase	0.786 (0.630—0.981)	0.033
	HCV RNA level	per 1-log increase	0.014 (0.000—0.918)	0.045
	ANA	ANA ≥1:80 = 1, ANA <1:80 = 0	0.014 (0.000-0.480)	0.018

Key: ANA = antinuclear antibody, ANA-positive = titers ≥1:80, ANA-negative = titers <1:80, HCV = hepatitis C virus, HCV-1 = with HCV genotype 1 infection, HCV-non 1 = without HCV genotype 1 infection, SVR = sustained virological response.

Table 6 Dose reduction of peginterferon/ribavirin and adverse events during treatment in 243 patients with chronic hepatitis C infection.

Feature	Patients (*n*, %)[a] *n*=243	ANA-positive *n*=14	ANA-negative *n*=229	*p*
Dose reduction				
Peginterferon	40 (16.5%)	2 (14.3%)	38 (16.6%)	1.000
Ribavirin	85 (35.0%)	4 (28.6%)	81 (35.4%)	0.776
Peginterferon or ribavirin	101 (41.6%)	5 (35.7%)	96 (41.9%)	0.783
Influenza-like symptoms				
Fever	165 (67.9%)	8 (57.1%)	157 (68.6%)	0.387
Chills	50 (20.6%)	5 (35.7%)	45 (19.7%)	0.172
Myalgia	138 (56.8%)	8 (57.1%)	130 (56.8%)	1.000
Headache	174 (71.6%)	11 (78.6%)	163 (71.2%)	0.762
Asthenia	154 (63.4%)	8 (57.1%)	146 (63.8%)	0.776
Gastrointestinal symptoms				
Anorexia	76 (31.3%)	5 (35.7%)	71 (31.0%)	0.769
Nausea	85 (35.0%)	5 (35.7%)	80 (34.9%)	1.000
Diarrhea	17 (7.0%)	1 (7.1%)	16 (7.0%)	1.000
Psychiatric symptoms				
Anxiety/depression	104 (42.8%)	7 (50.0%)	97 (42.4%)	0.590
Insomnia	145 (59.7%)	8 (57.1%)	137 (59.8%)	1.000
Dermatological symptoms				
Hair loss	131 (53.9%)	9 (64.3%)	121 (52.8%)	0.583
Skin rash	166 (68.3%)	11 (78.6%)	155 (67.7%)	0.558
Injection site erythema	88 (36.2%)	6 (42.9%)	82 (35.8%)	0.580
Body weight loss	44 (18.1%)	4 (28.6%)	40 (17.5%)	0.290
Anemia (hemoglobin <10 g/dL)	110 (45.2%)	8 (57.1%)	102 (44.5%)	0.414
Leucopenia				
White cell count <3000/mm^3	185 (76.15%)	12 (85.7%)	173 (75.5%)	0.528
White cell count <1500/mm^3	6 (2.5%)	1 (7.1%)	5 (2.2%)	0.302
Thrombocytopenia (<100 K/mm^3)	110 (45.3%)	6 (42.9%)	104 (45.4%)	1.000
Abnormal thyroid function tests	18 (7.4%)	0 (0.0%)	18 (7.9%)	0.608

[a] peginterferon/ribavirin for 24 weeks (*n* = 243).

an increase in interface hepatitis score. ANAs are associated with increasing age in both sexes [36]. The incidence of ANAs increases with age and the host factors of aging have also shown a strong association with fibrosis progression in HCV infection [37]. Thus, the pathogenesis and progression of liver damage might relate to host immunity and aging. In our previous study [14], we showed the association between ANA and the more advanced hepatic fibrosis, indicating that the evaluation of ANA as a marker of liver disease activity in patients with CHC might be important and helpful in predicting a more rapid progression of fibrosis.

The influence of NOSA on the efficacy of antiviral treatment has been controversial in previous reports [6—10]. Since HCV genotype is the most important factor associated with SVR, we stratified patients by genotype for further evaluation of the influence of ANA on treatment efficacy. After potential confounding factors were controlled, ANA titer $\geq$1:80 was inversely associated with the achievement of an SVR in HCV-non 1 patients, but not in HCV-1 patients. The mechanism for the association between high ANA titer and treatment response in HCV-non 1 patients remains unclear. In our previous study of 614 CHC patients, ANA seropositivity was associated with advanced fibrosis and older age [14], which are poor prognostic factors associated with treatment response. In the current study, as well as in previous studies [19,25—27,29—31], ANAs might be considered an indicator of the length of HCV infection, with a higher ANA titer (as a host factor) being considered a negative predictor of response to combination treatment in patients with HCV-non 1. In patients with HCV- 1, however, a stronger virology factor would cushion the affect of ANA.

The immunomodulatory effects of IFN make it important to consider the side effects and complications of the drug, such as the abnormal thyroid function we found in our previous studies [38,39]. The proportion of patients developing thyroid dysfunction in this study, however, was no higher (14.7% vs. 18.7%) than in our previous reports. In this study, none of our enrolled patients experienced a flare-up of serum ALT, and none of the seven patients who had high ANA titers ($\geq$1:160) suffered from a flare-up of serum ALT levels. The frequencies of adverse events were similar between patients with and without ANA seropositivity. These results suggest that the treatment of autoimmune hepatitis-unrelated CHC patients with high ANA titers is safe under close monitoring.

Conclusion

Our results demonstrate that PEG-IFN/ribavirin combination therapy is effective and safe in ANA-positive CHC patients for whom the major diagnosis of probable autoimmune hepatitis has been ruled out. High titer of ANA is a negative prognostic factor of treatment response in HCV-non 1 patients.

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